

Lipoprotein(a) and Apolipoprotein Changes After Cardiac Transplantation

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Although lipoprotein changes after cardiac transplantation have been documented, the effects of transplantation and subsequent immunosuppressive therapy (particularly the combination of prednisone, azathioprine and cyclosporine) on apolipoprotein levels and lipoprotein(a) have not been reported. Fasting cholesterol, triglycerides, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, apolipoprotein A-1 and B-100 and lipoprotein(a) were evaluated in 69 consecutive patients during the waiting period before cardiac transplantation.

There were 28 deaths before donor organ identification and 41 patients received a cardiac allograft. The lipoprotein levels of transplant recipients were again assayed 3 months postoperatively. Mean (\pm SEM) values increased for total plasma cholesterol (from 180 ± 8 to 228 ± 8 mg/dl, $p \leq 0.001$), triglycerides

(from 126 ± 11 to 207 ± 14 mg/dl; $p \leq 0.001$), HDL cholesterol (from 39 ± 2 to 49 ± 3 mg/dl; $p \leq 0.002$) and LDL cholesterol (from 119 ± 7 to 138 ± 7 mg/dl; $p < 0.02$). Apolipoprotein A-1 and B-100 also increased, but lipoprotein(a) decreased from 11.7 ± 1.7 to 6.8 ± 1.1 mg/dl; $p \leq 0.0001$) after transplantation.

Although total cholesterol, triglycerides, LDL cholesterol, apolipoprotein A-1 and B-100 increased dramatically after cardiac transplantation, so did HDL cholesterol, thereby keeping the LDL/HDL cholesterol ratio constant. The surprising decrease in lipoprotein(a) after cardiac transplantation suggests that metabolism of lipoprotein(a) is independent of LDL cholesterol and that immunosuppressive drugs either decrease the synthesis or increase catabolism of lipoprotein(a).

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Although cardiac transplantation is an accepted treatment for some patients with end-stage heart failure, the procedure still represents a sizable risk in terms of late outcome (1). Acute rejection may have decreased in relative importance, but accelerated obstructive vascular disease (sometimes referred to as chronic rejection) has become a major cause of posttransplantation death (2). The prevalence of this problem is high: some investigators (3) estimate that $>50\%$ of all patients undergoing cardiac transplantation who survive ≥ 3 years have obstructive vascular disease. Although multiple factors may play a role in this process, one of the most important seems to be an altered immune environment: classic cardiovascular risk factors are possibly less impor-

tant (4-6). Although the relative importance of hyperlipidemia in transplantation-associated atherosclerosis is controversial, it is not unreasonable to suspect that increased blood levels of atherogenic particles will act synergistically with immune factors to accelerate development of coronary artery disease (5).

Previous reports (7-11) have documented the presence of hyperlipidemia after cardiac transplantation, but alterations in various subfractions of circulating blood lipids after the procedure have not been well studied. Indeed, the effect of cardiac transplantation on some lipoprotein fractions such as lipoprotein(a), a lipoprotein highly correlated with coronary artery atherosclerosis, has not been reported. Recent studies (12,13) have demonstrated the importance of lipoprotein(a) as a marker for the presence and severity of coronary atherosclerosis in patients not undergoing transplantation.

The purpose of this study was to prospectively examine changes in total cholesterol, low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides and particularly apolipoproteins A-1, B-100 and lipoprotein (a) in patients undergoing cardiac transplantation. No prospective longitudinal evaluation of changes in all of these analytes has yet been reported.

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Methods

Study subjects. Patients were evaluated in our institutions with the use of protocols previously described (1). A total of 69 patients were prospectively studied before undergoing cardiac transplantation. The patients were identified with respect to gender, age and origin of congestive heart failure. All patients were included in a serial manner from March 1988 to May 1989. A total of 28 patients died before receiving a cardiac transplant and were then excluded from further data analysis. Medication lists were obtained at each clinic visit in the posttransplantation period. Lipid values were tabulated serially and values obtained 6 weeks before transplantation were compared with those measured 3 months after transplantation. The mean (\pm SEM) time interval between lipid analysis and transplantation was 40 ± 13 days; all but one patient had baseline lipid levels measured within 5 months of transplantation.

All postoperative patients received triple-drug immunosuppressive therapy with azathioprine, cyclosporine and prednisone. Monoclonal antibody therapy (OKT3) was not used for rejection prophylaxis, but rather for rejection treatment. Mean daily doses for all patients at 3 months were 20 mg of prednisone, 7.2 mg/kg body weight of cyclosporine and 65 mg/day of azathioprine. None of the patients received antithymocyte globulin. Routine antibiotic therapy was administered in the form of intravenous cefoxitin or cefuroxime and this treatment was continued until removal of chest tubes. The average duration of routine antibiotic therapy was 72 h.

Lipid determinations. Blood samples were obtained after a 14-h fast. The samples were anticoagulated with ethylenediaminetetraacetic acid (EDTA) and plasma was separated by low speed centrifugation. The plasma concentration of cholesterol, triglycerides and HDL cholesterol was measured according to methods outlined previously (7). LDL cholesterol was calculated by the standard formula: $\text{LDL cholesterol} = \text{Total cholesterol} - ((\text{HDL cholesterol} + \text{Triglycerides})/3)$ (14). Because triglyceride levels were consistently <400 mg/dl, this was believed to be an accurate extrapolation (14).

The levels of lipoproteins were determined by an enzyme-linked immunosorbent assay (ELISA) utilizing a method previously described (15). Rabbits were immunized with lipoprotein(a) and an antiserum that was nonspecific for lipoprotein(a) was prepared. The rabbit antilipoprotein(a) serum was adsorbed with human LDL and the resultant LDL antiapolipoprotein B precipitate was removed. The supernatant yielded a single precipitin line with lipoprotein(a), but no reaction with LDL on Ouchterlony gels. Patient samples were stored at 4°C and analyzed within 48 h. Purified lipoprotein(a) was isolated from the plasma of patients with high levels of this lipoprotein. After measurement of its protein content and immunoreactivity, this purified lipoprotein was used as a primary reference for standardizing plasma samples containing low, medium and high

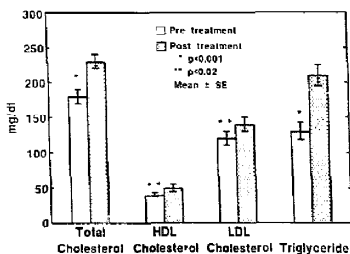


Figure 1. Changes in serum total, high density lipoprotein (HDL) and low density lipoprotein (LDL) cholesterol and triglycerides after cardiac transplantation in 41 patients. Post = after; Pre = before.

lipoproteins) levels, which were then used as secondary standards. The standard dilution curve was linear, with a correlation coefficient of 0.90. Intra- and interassay coefficients of variation were 4% and 9%, respectively. For normal lipoprotein(a) protein levels in the range of 1 to 10 mg/dl, the contribution of plasminogen at physiologic concentration (200 mg/dl) was negligible (16).

Statistical evaluations. Lipoprotein values were arranged according to pre- and posttransplantation groups and reported as mean values \pm SEM. The difference between values in each group was analyzed using paired *t* tests and significance was assigned at a *p* value < 0.05 . Linear regression analysis was utilized to compare changes in lipoprotein(a) with changes in other lipoprotein values.

Informed consent. The study protocol was approved by the Institutional Review Board of Baylor College of Medicine, The Methodist Hospital and St. Luke's Episcopal Hospital. All patients gave informed consent before enrollment in the study.

Results

Patient characteristics. Of the patients entered into the study, 41 underwent cardiac transplantation and were subsequently reevaluated. There were 34 men (82%) and 7 women (17%), ranging in age from 23 to 67 years (mean \pm SD 51 ± 9.7). The racial distribution was 36 white (88%), 4 Hispanic (10%) and 1 black (2%). The underlying cause of congestive heart failure treated by transplantation was ischemia in 29 patients (71%), idiopathic in 6 (14%), viral in 2 (5%), valvular in 2 (5%), congenital or other in 1 each (2%).

Cholesterol levels. Figure 1 summarizes the changes we observed in total, HDL and LDL cholesterol levels in the transplant recipients. The mean total cholesterol level before transplantation was 180 ± 8 mg/dl; after transplantation, it increased to 228 ± 8 mg/dl ($p \leq 0.001$). Mean triglycerides

Table 1. Changes in Apolipoproteins After Transplantation in 41 Patients

	Pre	Post	p Value
Apo A-I (mg/dl)	86 ± 4	105 ± 4	<0.002
Apo B-100 (mg/dl)	83 ± 4	101 ± 5	<0.008
Apo B-100/apo A-I	0.97	0.96	NS

Apo = apolipoprotein; Post = after transplantation; Pre = before transplantation.

also changed dramatically (126 ± 11 mg/dl and 207 ± 14 mg/dl, respectively, before and after transplantation; ($p \leq 0.001$). HDL cholesterol increased from 39 ± 2 to 49 ± 3 mg/dl postoperatively ($p < 0.002$) and LDL cholesterol level from 119 ± 7 to 138 ± 7 mg/dl postoperatively ($p < 0.02$).

Apolipoprotein levels (Table 1). The LDL/HDL cholesterol ratio did not change significantly because both HDL and LDL cholesterol increased proportionately. The ratio of 3.4 before transplantation decreased only slightly after transplantation to 3.3 ($p > 0.05$). The apolipoprotein A-I level was 86 ± 4 mg/dl before transplantation and increased to 105 ± 4 mg/dl ($p < 0.002$) afterward. The apolipoprotein B-100 level was 83 ± 4 mg/dl before transplantation, increasing to 101 ± 5 mg/dl ($p < 0.008$) afterward. The ratio of apolipoprotein B-100/apolipoprotein A-I did not change.

Lipoprotein levels. Changes in lipoprotein(a) protein levels were analyzed by several statistical methods. For the entire group, the mean lipoprotein(a) level of 11.7 ± 1.7 mg/dl before transplantation (Fig. 2) decreased to 6.8 ± 1.1 mg/dl after transplantation ($p < 0.001$). On further examination, a total of 28 patients had a decrease >1 mg/dl for lipoprotein(a) protein after transplantation. The range for the decline was 1 to 22.5 mg/dl. A total of 13 patients had essentially no change (<1 mg/dl decrease) or an increase;

(range of difference 0 to -0.9 mg/dl). The changes in lipoprotein(a) were independent of changes in total cholesterol, triglycerides, HDL cholesterol or LDL cholesterol when examined by regression analysis.

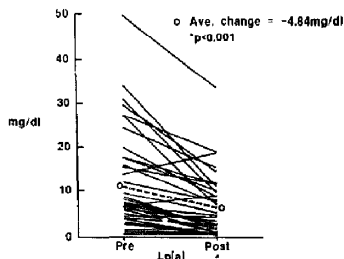
Discussion

Changes in lipid and lipoprotein levels after cardiac transplantation. This study confirms and extends earlier observations (7-11) that cardiac transplantation causes significant changes in the lipid and lipoprotein profile. Those changes are multifactorial in origin. All patients in our study had severe congestive heart failure with New York Heart Association class III or IV symptoms before transplantation. Many patients had abnormally low cholesterol values before transplantation. Thus, correction of heart failure with concomitant correction of hemodynamic abnormalities and improved nutritional status may have contributed to the increase in plasma lipid concentration occurring after transplantation. In addition, each patient received immunosuppressive drug therapy consisting of azathioprine, cyclosporine and prednisone after transplantation. Several previous studies have examined the effects of cyclosporine and corticosteroids on lipids. Cyclosporine raises total cholesterol primarily by increasing LDL cholesterol (17). Corticosteroids, which also raise total cholesterol levels, produce this effect primarily by elevating very low density lipoprotein (VLDL) cholesterol and HDL cholesterol (18,19). Our observed significant increase in plasma triglycerides reflects an increase in VLDL cholesterol. The increase in apolipoprotein A-I was accompanied by a parallel marked increase in HDL cholesterol. Alger et al. (20) observed that an increase in HDL cholesterol is due primarily to an increase in cholesterol within HDL₂, the lipoprotein subfraction that has the greatest negative correlation with coronary artery disease.

The posttransplantation elevation of HDL cholesterol was attended by an increase in both LDL cholesterol and the major LDL apolipoprotein B-100. Baseline data before transplantation revealed an LDL/HDL cholesterol ratio of 3.4, which was not significantly changed after transplantation. Hence, the relative risk of coronary artery disease, as evaluated by this criterion, was not changed appreciably by transplantation. Likewise, similar increases were observed in mean plasma levels of apolipoproteins A-I and B-100, such that their mean ratios were not significantly changed.

Effect of transplantation on lipoprotein(a) level: role of drugs. In striking contrast to other lipids or apolipoproteins measured in this study, the mean level of lipoprotein(a) protein decreased by $>40\%$ during the follow-up period after cardiac transplantation. This remarkable decrease in the setting of significant increases in LDL cholesterol and apolipoprotein B-100 provides strong support for the view that a major fraction of lipoprotein(a) is metabolized through a pathway distinctly different from that followed by LDL (21). Although a number of our patients altered their dietary

Figure 2. Changes in lipoprotein(a) (Lp[a]) after cardiac transplantation in 41 patients. Ave. = average; other abbreviations as in Figure 1.



habits and one began a lipid-lowering drug regimen after cardiac transplantation, it is unlikely that these maneuvers significantly lowered their mean lipoprotein(a) level. All patients received instructions in the American Heart Association step 1 diet. Although decreased intake of dietary cholesterol may cause a decrease in plasma LDL cholesterol, very little effect has been observed on lipoprotein(a) (21,22). Furthermore, no patient was taking niacin, the only lipid-lowering drug that has been shown to lower lipoprotein(a) (23). One patient was taking lovastatin (20 mg/day), a drug that does not lower lipoprotein(a) (24). There was no significant change in this patient's lipoprotein(a) value (2.4 mg/dl before transplantation to 1.2 mg/dl after transplantation). Significantly, lipoprotein(a) increases rapidly and markedly after acute myocardial infarction or surgery, in concert with other acute phase-reactant proteins such as C-reactive protein (25). Apparently, this elevating effect had subsided by the time our patients had blood samples redrawn for their second battery of lipid tests.

Hence, we are led to propose that it is primarily the immunosuppressive drugs that lower lipoprotein(a) levels in patients after transplantation. Drugs such as cyclosporine and prednisone, which interfere with the cascade of cellular immune activation, and cytokines, which lead to inflammation, might inhibit lipoprotein(a) synthesis. Previous studies of lipoprotein(a) metabolism suggest other mechanisms whereby cyclosporine or prednisone could affect this lipoprotein. Because lipoprotein(a) levels decrease in patients with hepatobiliary disease (26), cyclosporine may have an effect on lipoprotein(a) synthesis that is associated with this drug's hepatotoxicity. Studies in the European hedgehog (27) have shown an inverse relation between plasma concentrations of lipoprotein(a) and the steroid hormone testosterone; anecdotal reports (28) suggest that anabolic steroids are associated with depressed levels of lipoprotein(a) in humans. Therefore, corticosteroids may have a comparable effect on lipoprotein(a) metabolism. The patients received no other drugs that have been shown conclusively to decrease lipoprotein(a).

Another possible mechanism whereby circulating plasma lipoprotein(a) might be depressed after transplantation could involve enhanced sequestration of the lipoprotein by an immune-mediated perturbation of the coronary endothelium. If this mechanism is operative, reduced levels of lipoprotein(a) would be associated with its increased deposition in the arterial wall, with subsequently reduced fibrinolysis, an enhanced likelihood of thrombosis and subsequent myocardial infarction.

Clinical implications. The effects of cardiac transplantation and the ensuing immunosuppression on lipoprotein(a) levels are striking. Indeed, the mean lipoprotein(a) level for our patient group was decreased by 50% at 3 months after transplantation. This change is not correlated with a concomitant change in LDL cholesterol. Although the plasma concentration of lipoprotein(a) is highly correlated with the presence and extent of coronary artery disease (13), the

clinical impact of lowering the lipoprotein(a) concentration in the setting of either acute or chronic disease has not been determined, nor has it been demonstrated that an induced decrease in lipoprotein(a) is associated with an alteration in the atherosclerotic process or the clinical course of the disease. Further studies are needed to elucidate the mechanisms whereby heart transplantation alters lipoprotein(a) levels and the clinical significance of this effect.

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